Phosphole *P*-Oxide-Containing π -Electron Materials: Synthesis and Applications in Fluorescence Imaging

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Abstract: Phosphole *P*-oxide is a useful building block for π -conjugated materials due to its nonaromatic and electron-accepting character. We have synthesized a series of ring-fused derivatives of phosphole *P*-oxide based on the intramolecular nucleophilic cyclization of appropriate alkyne precursors or radical phosphanylations. Some of the thus obtained compounds exhibited intriguing fluorescence properties and were applied to fluorescence imaging. A donor-acceptor-type benzo[*b*]phosphole *P*-oxide with a (diphenylamino)phenyl group exhibited large solvatochromism in its fluorescence spectra, and could hence be used as a staining agent for lipid droplets. C-Naphox and PB430, which consist of fully ring-fused π -conjugated ladder-type scaffolds, exhibited outstanding photostability and their absorption and emission properties were suitable for super-resolution STED imaging. Moreover, using PB430-conjugated antibodies, we carried out a 3-D reconstruction of the STED images and developed a photostability-based multicolor STED imaging technique.

1. Introduction

Molecular design based on the exploitation of specific properties of main-group elements represents a powerful strategy to produce useful π -electron materials with characteristic electronic structures.¹ Among various main-group elements, phosphorous is particularly useful to this end.² The introduction of phosphorus moieties into π -conjugated cyclic skeletons produces highly useful building blocks for π -electron materials. Moreover, simple chemical transformations from phosphanes to phosphonium salts, phosphine oxides or sulfides, as well as complexation to transition metals significantly alters their electronic properties.

For such π -conjugated skeletons, the five-membered ring skeletons, *i.e.*, phospholes, are very important.² Even though phospholes represent heavier analogues of pyrrole, the intrinsic character of these homologues is very different. While pyrrole is an electron-donating aromatic ring, phosphole acts as a nonaromatic cyclic diene, owing to the fact that the lone pair of electrons on phosphorus does not participate readily in the π -conjugation. Therefore, phospholes exhibit a relatively low-lying LUMO compared not only to pyrrole, but also to other heterole rings (Figure 1).³ Importantly, this characteristic feature is enhanced by the oxidation of the phosphorus atom to the phosphine oxide or sulfide. Phosphole *P*-oxides or *P*-sulfides are hence useful electron-accepting moieties. Although phosphonium moieties are also highly electron-accepting, their relatively low chemical stability somewhat decreases their utility as scaffolds for π -electron materials. Based on these considerations, phosphole *P*-oxides and *P*-sulfides have attracted considerable attention, and a number of fascinating π -electron systems has been developed using these scaffolds.² Most of these compounds have been studied with respect to their applications in organic electronics,⁴ including organic light emitting devices (OLEDs),⁵ thin-film transistors,⁶ and

photovoltaic cells.⁷ However, their utility should not be limited to such applications. In light of the highly electron-accepting character in combination with their high chemical stability, these building blocks should exhibit significant potential as core scaffolds for biological applications, as *e.g.* fluorescence probes for bioimaging.⁸

Figure 1. a) Comparison of the electronic structure of pyrrole, phosphole, and phosphole *P*-oxide based on calculations at the B3LYP/6-31G(d) level of theory, and b) benzo[*b*]phosphole as the focus of this review.

Fluorescence probes have become indispensable tools in contemporary biological research for the *in vitro* and *in vivo* visualization of individual biomolecules. The progress of this area relies not only on the advancement of microscopy techniques, but also on that of the fluorescence dyes. The former have been significantly advanced during the last decade, exemplified by the development of super-resolution microscopy methods, such as stimulated emission depletion (STED) microscopy. Although various fluorescent molecules have been developed,⁹ a similar technological leap has not yet been achieved for the fluorescent dyes employed, particularly in terms of photostability, which is one of the most important properties for such dyes.¹⁰

In this context, we have demonstrated that phosphole *P*-oxides are highly useful, as they produce highly photostable

dyes. In particular, we have focused our attention on the benzo-fused phosphole skeleton, benzo[*b*]phosphole *P*-oxide, to produce stable fluorescence dyes. In this article, we offer a concise summary on the progress of our research with regard to the synthesis and modification of such ring-fused phosphole skeletons, and their subsequent applications in fluorescence bioimaging.

2. Synthetic Routes to Ring-Fused Phosphole *P*-Oxides

2.1 Intramolecular Bisphosphanylation of Alkynes

Various synthetic methods have been reported for the construction of benzo[*b*]phosphole-based π -electron materials. Those methods can be classified into: i) a cross-coupling reactions using halogenated benzo[*b*]phospholes,¹¹ ii) intermolecular cycloadditions of alkynes and arylphosphines,¹² iii) reactions that proceed via dimetallated alkenylarene intermediates with phosphorus reagents,¹³ and iv) intramolecular cyclizations of alkynylarenes with *o*-phosphorus groups.¹⁴ Our approach to construct the benzophosphole skeleton is based on a type-iv reaction (Scheme 1).¹⁵ It is particularly noteworthy that this approach enables the construction of a bis (phosphine oxide)-bridged stilbene skeleton in one pot from bis (*o*-bromophenyl)-acetylene **1**.

Scheme 1. Intramolecular double cyclization to produce bis(P=O)-bridged stilbenes **3a,b**.

Bis[2-(amino-phosphanyl)phenyl]acetylene **2** was initially generated *in situ* from **1**. Without isolation, **2** was subsequently treated with PCl_3 , which efficiently produced P=O-bridged stilbenes **3a,b**. In this reaction, one of the phosphanyl groups acts as a nucleophile, and the other as an electrophile; consequently, the *unsymmetrically* occurring cascade cyclization produces *symmetrically* bridged stilbenes.

The resulting P=O-bridged stilbenes are obtained as two geometrical *cis* (**3a**) and *trans* (**3b**) isomers, which differ in terms of their dipole moment and steric congestion, and may thus find different applications. These P=O-bridged stilbenes also show unusual luminescence properties, which are significantly different from those of other stilbene analogues bearing carbon or silicon-bridges,¹⁶ especially in terms of the absorption and fluorescence wavelengths ($\lambda_{\text{max}} = 395 \text{ nm}$, $\lambda_{\text{em}} = 480 \text{ nm}$ in CH_2Cl_2), the fluorescence quantum yields (**3a**, $\Phi_{\text{F}} = 0.99$), and the excited-state dynamics. The P=O-bridges dramatically enhance the electron-accepting ability of **3a,b**, which is reflected in reversible one-electron reduction waves [$E_{1/2} = -1.63 \text{ V}$ and -1.67 V vs. ferrocene/ferrocenium (Fc/Fc^+) for **3a** and **3b**, respectively] in the cyclic voltammograms measured in THF. This result suggests potential use of **3a,b** as

an electron-accepting scaffold for donor-acceptor (D-A) - type molecules.

2.2 Intramolecular Phosphaborylation of Alkynes

The key driving force for the intramolecular double phosphanylations presented in the previous section is the sufficiently high nucleophilicity of the phenylphosphanyl group. Therefore, the electrophilic moiety should be replaced. Based on this idea, this synthetic method was successfully extended to the preparation of phosphonium and borate-bridged stilbenes **5**. Following the *in situ* generation of [*o*-(dialkylphosphanyl)phenyl][*o*-(dimesitylboryl)phenyl]acetylene **4a,b**, a spontaneous reaction furnished zwitterionic π -conjugated **5a,b**.¹⁷ When a diphenylphosphanyl group is used instead of the dialkylphosphanyl group (**4c**), the reaction does not proceed even at elevated temperatures. However, the double cyclization occurs under photoirradiation.¹⁸ Since this reaction is not reversible, this system is not photochromic. However, interestingly, this photochemical cyclization is accompanied by a significant color change. Moreover, this synthetic method can be used for the synthesis of a series of compounds **6** with more extended π -conjugation (Figure 2), which exhibit attractive photophysical properties that include a large two-photon absorption cross section.¹⁹

Scheme 2. Intramolecular double cyclization of **4a-c** to produce phosphonium and borate-bridged stilbenes **5a-c**.

Figure 2. Phosphonium and borate-bridged compounds **6** with extended π -conjugation.

2.3 Intramolecular *trans*-Halosphosphanylation

The importance of the nucleophilicity of the phosphanyl group for the intramolecular phosphanylation can also be observed for similar intramolecular monocyclizations that produce a benzo[*b*]phosphole skeleton (Scheme 3).²⁰ *o*-(Aminophosphanyl)-substituted phenylacetylene **7** undergoes an intramolecular cyclization upon treatment with PBr_3 . This reaction is triggered by the halogenation of the aminophosphane. It is noteworthy that the generated halophosphanyl

group undergoes a *trans*-halophosphanylation to the alkyne moiety. Although the detailed mechanism of this reaction remains unclear, it should have significant synthetic value, as the product is halogenated benzo[*b*]phosphole **8**, from which various 3-substituted benzophosphole π -electron materials can be easily obtained.

Scheme 3. Intramolecular *trans*-bromophosphanylation to afford **8**.

Among these derivatives, phosphoryl- and methylene-bridged stilbene **9** is of particular interest. This compound exhibits absorption ($\lambda_{\text{abs}} = 367$ nm) and emission maxima ($\lambda_{\text{em}} = 443$ nm) with a high quantum yield ($\Phi_F = 0.85$). These values are slightly shorter than those for bis(P=O)-bridged **3**. This result clearly demonstrates that the introducing of the P=O moiety induces a bathochromic shift of the absorption and emission maxima. The electron-withdrawing properties and the $\sigma^*-\pi^*$ orbital interactions of the P=O moiety decrease the LUMO energy level, which results in a decreased $\pi-\pi^*$ transition energy.

The photophysical properties of **9** also provide important insight into the impact of the fully ring-fused skeleton. In general, the extent of π -conjugation increases upon increasing the coplanarity of the π -skeleton, which results in a bathochromic shift of the absorption and emission maxima, together with an increase of the molar absorption coefficient (ϵ). However, doubly bridged **9** exhibits smaller values for ϵ and the radiative rate constant k_r ($\epsilon = 6.7 \times 10^3$; $k_r = 7.9 \times 10^7$) compared to those of the singly bridged **10** ($\epsilon = 9.3 \times 10^3$; $k_r = 1.4 \times 10^8$). These results are counter-intuitive. This discrepancy should be attributed to the effect of the deformation in the parent stilbene skeleton. The theoretically optimized structures for **9** and **10** show that doubly bridged **9** exhibits smaller C3-C4-C5 and C4-C5-C6 angles than singly-bridged **10** (Figure 3). TD DFT calculations revealed that this struc-

Figure 3. Superimposed stilbene substructures of mono- and bis-bridged stilbene derivatives **9** and **10**, based on theoretically optimized structures calculated at the B3LYP/6-31G(d) level of theory.

tural deformation results in a decrease of the oscillator strength, and hence a decrease of the ϵ and k_r values. This is an important effect of the ring-fusion in such five-membered rings, that can be the basis of the molecular design for super-photostable fluorescent dyes (*cf.* section 4).

2.4 Radical Phosphanylation

The formation of the C-P bond is a key issue for any synthesis of phosphorus-containing π -electron materials. In this context, Studer and co-workers have reported the useful radical phosphanylation of aryl halides.²¹ We employed their method for the synthesis of bis(P=O)-bridged biphenyl **12** (Scheme 4).²² We treated 2,2,2',2'-tetrabromobiphenyl (**11**) with $(\text{Me}_3\text{Sn})_2\text{PPh}$ in the presence of the initiator 1,1'-azobis(cyclohexane-1-carbonitrile) (V-40). The phosphanylation proceeded in benzonitrile at 125 °C, and prolonged reaction times led to higher conversions. After oxidation with H_2O_2 , *trans* (**12a**) and *cis* isomers (**12b**) were isolated in good yields. It is worth noting that benzonitrile effectively reduces the reaction period, and that the four-fold radical phosphanylation in this transformation proceeds in acceptable yield, despite the severe ring strain in the final step. This result is a good demonstration of the efficiency of this radical phosphanylation.

Scheme 4. Synthesis of bis(P=O)-bridged biphenyls **12** by radical phosphanylation.

The thus produced bis(P=O)-bridged biphenyls **12a,b** should be useful electron-accepting building blocks. An intriguing derivative that we have synthesized is diphenylaminophenyl-substituted D-A-D type compound **13** (Figure 4), which exhibited absorption and emission maxima at 399 nm and 547 nm in CH_2Cl_2 , respectively, in combination with a moderate quantum yield ($\Phi_F = 0.30$). We have also synthesized more compact D-A-D compound **14**, which connects dibenzylamino groups directly to the biphenyl core (Figure 4).²³ This compound shows $\lambda_{\text{abs}} = 464$ nm with a relatively small absorption coefficient ($\epsilon = 870$) and an orange emission with $\lambda_{\text{em}} = 594$ nm in CH_2Cl_2 , which is notably longer than that of **13**.

Figure 4. D-A-D type biphenyls **13** and **14** with double P=O bridges.

Notably, this radical phosphanylation is a robust method to construct fused phosphole skeletons. This approach could even be successfully applied to tetrabromopyridine, which resulted in the formation of a bis(P=S)-bridged bipyridine,²⁴

which was further converted into dimethylated viologen-type **15**. This compound showed outstanding electron-accepting properties. In CH₃CN, the cyclic voltammogram of **15** exhibited two fully reversible redox processes for the reduction ($E_{\text{red1,1/2}} = -0.41$ V; $E_{\text{red2,1/2}} = -0.91$ V vs. Fc/Fc⁺) (Figure 5). A similar mono(P=O)-bridged viologen has been intensively studied by Baumgartner and co-workers.²⁵

Figure 5. Cyclic voltammogram of bis(P=S)-bridged viologen **15** (1 mM in MeCN with [*n*-Bu₄N][PF₆] vs. Fc/Fc⁺).

3. Benzo[*b*]phosphole-Based Fluorescence Dyes

3.1 Fluorescence Properties of Donor-Acceptor-Type Benzophosphole *P*-Oxides

Based on the previously established phosphole synthesis, we have synthesized various types of benzo[*b*]phosphole *P*-oxide derivatives, some of which showed intriguing fluorescence properties. A representative example is the class of completely rigid bis(P=O)-bridged derivatives, which show intense blue fluorescence with a quantum yield of unity. The other crucial feature of the benzo[*b*]phosphole *P*-oxide skeleton is its electron-accepting character. When an electron-donating group is introduced, the resulting D-A-type molecule can exhibit intense fluorescence. In addition, the fluorescence of such molecules is subject to large solvatochromism. Indeed, diphenylaminophenyl-substituted benzo[*b*]phosphole *P*-oxide **16** exhibited intriguing fluorescence properties.³

In toluene, **16** exhibited an absorption band with $\lambda_{\text{max}} = 415$ nm, while an intense fluorescence band was observed at $\lambda_{\text{max}} = 528$ nm ($\Phi_F = 0.94$). With increasing solvent polarity, the absorption spectra showed subtle changes ($\lambda_{\text{abs}} = 403\text{--}420$ nm), while the fluorescence band was significantly red-shifted (DMSO: $\lambda_{\text{max}} = 601$ nm; EtOH: $\lambda_{\text{max}} = 593$ nm) (Figure 6). In general, D-A-type molecules that exhibit

Figure 6. Solvent-dependent absorption and fluorescence spectra of **16**.

such large solvatochromism tend to show significantly decreased fluorescence quantum yields in polar solvents. Notably, **16** can retain high quantum yields even in polar (DMSO: $\Phi_F = 0.64$) or protic solvents (EtOH: $\Phi_F = 0.58$) despite its large Stokes shifts (DMSO: 7,630 cm⁻¹; EtOH: 7,120 cm⁻¹).

To attain this desirable property, the well-balanced combination of the electron-accepting phosphole *P*-oxide and the electron-donating diphenylamino groups in **16** is crucial, which is evident by comparisons of **16** with related compounds, including benzophosphole *P*-sulfide **17**, phosphonium salt **18**, and thiophene dioxide congener **19** (Figure 7). In terms of the LUMO levels, the parent phosphole rings with P=O or P=S moieties possess comparable electron-accepting properties. Accordingly, the photophysical properties of phosphole *P*-oxide **16** and phosphole *P*-sulfide **17** are comparable, *i.e.*, **17** exhibits high fluorescence quantum yields both in polar (DMSO: $\Phi_F = 0.61$) and in protic (EtOH: $\Phi_F = 0.76$) solvents with similar maximum emission wavelengths to those of **16**. In contrast, the electron-accepting character of the phosphole phosphonium ring in **18** is much stronger than the phosphole ring with a P=O moiety in **16**. Consequently, **18** exhibits a bathochromically shifted absorption ($\Delta\lambda = 33$ nm) and fluorescence maxima ($\Delta\lambda = 144$ nm) relative to those of **16**, even in toluene. Associated with this difference, **18** shows substantially lower Φ_F values irrespective of the solvent polarity. Compound **19**, which also contains a stronger electron-accepting moiety than **16**, exhibits properties similar to those of **18**.

Figure 7. Photophysical properties of a series of D-A-type benzophospholes **16**–**18** and a related compound **19**.

The position of the electron-donating group is also crucial for the fluorescence properties of **16**: although structural isomer **20** bears an identical aminophenyl group at the 3-position, its behavior is markedly different.²⁶ While **20** shows only a subtle dependence of the absorption maximum on the solvent polarity ($\lambda_{\text{max}} = 383\text{--}392$ nm), its emission maximum is significantly red-shifted upon increasing the solvent polarity (cyclohexane: $\lambda_{\text{em}} = 457$ nm; DMF: $\lambda_{\text{em}} = 598$ nm). Most notably, the quantum yield gradually increases with increasing solvent polarity, to ultimately reach $\Phi_F = 0.28$ in DMF (Figure 8), despite being accompanied by increasing Stokes shifts.

The origin of this unusual phenomenon was studied in detail by experimental and theoretical studies, whereby a particular focus was placed on the calculation of the first excited singlet state (*S*₁) at the TD-CAM-B3LYP/6-31G(d) level of

Figure 8. Solvent-dependent fluorescence quantum yields of **16** and **20**.

theory.²⁶ The results revealed that the electron-donating group at the 3-position of the benzophosphole ring influences the excited state through a substantial contribution of a quinoidal resonance structure, as a consequence of the intramolecular charge-transfer transition. This is noteworthy, as 3-aryl groups are generally considered to refrain from a significant participation in the π -conjugation of heterole or benzohe-terole rings.

3.2 Applications of Benzo[*b*]phosphole *P*-Oxide **16** as a Fluorescence Probe for Lipid Droplets

Environment-sensitive fluorescence probes are important for the visualization of polarity changes associated with cellular events,²⁷ and several suitable fluorophores, such as prodan, 1,8-ANS, dapoxyl, and Nile red, have been reported. In comparison to these, D-A-type benzophosphole *P*-oxide **16** offers some attractive advantages that include i) compatibility with light-emitting diode lasers ($\lambda = 405$ nm), which are frequently used for excitation in fluorescence microscopy, and ii) red fluorescence emission as a result of the excitation at 405 nm. This fact prompted us to examine the potential of **16** as a fluorescence probe.³

Fluorescence imaging of differentiated 3T3-L1 adipocytes with **16** was investigated using a confocal microscope equipped with a GaAsP multi-channel spectral detector (Figure 9). When 3T3-L1 cells were incubated with 1 μ M of **16** in Dulbecco's modified Eagle's medium (DMEM) for 2 h at 37 °C, a diffuse staining pattern with several bright spots appeared (0 day, column a), demonstrating that **16** is membrane permeable. The fluorescence spectra of the bright spots showed an emission maximum at 521 nm, whereas the emission spectrum in other intracellular domains exhibited emission maxima at a longer wavelength (565 nm). Using these spectral properties, the image was unmixed into two components shown in green (column b) and orange (column c). Based on this procedure, the fluorescent solvatochromism of **16** allowed us to discriminate different environmental polarities in the cells. As the differentiation of 3T3-L1 cells into adipocytes proceeded, **16** is dissolved in the hydrophobic lipid droplets, which allowed visualizing their polarity. Meanwhile, the emission intensities in the other domains gradually decreased during the adipogenic differentiation (column c), which may correspond to a concentration decrease of filamentous actin (F-actin). Throughout this measurement, **16** showed high photostability, which is superior to some representative dyes, such as BODIPY, fluorescein, and prodan. In addition, the cytotoxicity of **16** is very low. In light of these features, **16** represents a useful reagent to stain adipocytes, and is now

Figure 9. Confocal-microscopy-based monitoring of the differentiation of 3T3-L1 preadipocytes with **16**. Cells were stained with **16** for 2 h prior to examination: a) lambda stack images for $\lambda_{em}=416-689$ nm with $\lambda_{ex}=405$ nm; b) and c) linear unmixing images using reference spectra of selected pixels.

commercially available under the brand name LipiDye.

3.3 Applications of Benzo[*b*]phosphole *P*-Oxides as Na⁺ Fluorescence Probes

Using the D-A-type benzophosphole oxide skeleton, we have also developed the new ratiometric probe NaGY by incorporating a diazacrown ether moiety, which serves both as a sodium ion-binding site and as an electron-donating moiety.²⁸ Sodium ions play essential roles in the regulation of signal transactions. Although several commercially available fluorescent sodium ion probes, such as SBF1 and CoroNa Green, have been widely employed, these probes still suffer from certain drawbacks in terms of *e.g.* absorption characteristic and low fluorescence turn-on ratio. In this context, a new fluorescent sodium ion probe that enables ratiometric detection and visible-light excitation is highly demanded. NaGY showed an absorption band in the visible region both before and after complexation with a sodium ion, together with red fluorescence ($\lambda_{em} = 656$ nm). NaGY also showed a hypsochromic shift ($\Delta\lambda = 36$ nm) of its fluorescence upon binding to the sodium ion (Figure 10). A membrane-permeable form, NaGY-AM, was synthesized and successfully used for the ratiometric analysis of the sodium ion influx in living mammalian cells.

Figure 10. Change of the fluorescence spectra of NaGY (25 μ M) in 50 mM HEPES (pH=7.4) ($\lambda_{ex}=405$ nm) upon addition of NaCl (0, 5, 10, 20, 40, 65, 100, or 200 mM).

4. Super-Photostable Fluorescent Dyes for STED Imaging

4.1 D-A-Type Photostable Dye C-Naphox

The most common and significant problem in contemporary fluorescence imaging is undoubtedly the photobleaching of the fluorescence dyes, especially in STED microscopy, as mentioned in the introduction. The development of STED microscopy represents a major breakthrough in the field of cellular and molecular biology, as STED allows the visualization of structural details beyond the optical resolution of conventional confocal microscopy.⁹ However, the intense laser beams required for both excitation and STED usually cause rapid photobleaching of the fluorescent molecular probes, which significantly limits the performance and practical utility of STED microscopy. For instance, the optimization of experimental conditions for STED imaging is rendered rather difficult, due to significant photobleaching of the fluorescence dyes during repeated observations. In the last decade, microscope technology for STED imaging has rapidly advanced from CW STED to gSTED and 3-D STED (commercially available from Leica Microsystems since 2013). Unfortunately, a similar technological leap regarding the photostability of the employed fluorescent dyes has not been accomplished during the same period. Accordingly, the development of fluorescent dyes with significantly increased photoresistance is highly desirable in order to fully exploit the potential of state-of-the-art STED microscopy.¹⁰

Although **16** exhibits a relatively high photostability, it is not sufficient for utilization in STED microscopy. Therefore, we tried to develop a skeleton with higher photostability using benzo[*b*]phosphole *P*-oxide as the core scaffold. Based on a structure-property-relationship study of a series of benzo-phosphole derivatives, we finally discovered that the fully ring-fused benzo[*b*]phosphole *P*-oxide-based π -conjugated compound C-Naphox (Figure 11) showed significantly improved photostability.²⁹ It should be noted that in C-Naphox, a naphthalene skeleton is used instead of a benzo-fused structure in order to accomplish the photophysical properties required for STED microscopy. Namely, the absorption properties of C-Naphox are suitable for photoexcitation with common visible light lasers (λ_{ex} = 405 or 488 nm). Apart from displaying bright fluorescence in polar solvents, C-Naphox also exhibits a large Stokes shift, which is advantageous in order to avoid auto-fluorescence interference and anti-Stokes excitation generated by the depletion laser.

Figure 11. Photostability of C-Naphox and other representative photostable dyes. Absorption variation of DMSO/HEPES buffer solutions (pH = 7.3; v/v=7/3) of C-Naphox, Alexa 488, and Atto 488 as a function of irradiation time with a Xe lamp (300 W) using a band-pass filter (460±11 nm).

Even compared to Alexa fluor 488 and Atto 488, which are representative photostable dyes that are widely used in STED imaging, the photostability of C-Naphox is outstanding (Figure 11). After irradiation with a Xe lamp (300 W) equipped with a band-pass filter (λ_{ex} = 460 nm; FWHM: 11 nm) for 2 h, 99.9% of C-Naphox remained intact, while only 26.2% of Alexa 488 and 96.7% of Atto 488 persisted. Even after 12 h of irradiation, C-Naphox still remained almost quantitatively intact (99.5%), while Atto 488 (58.7%) suffered from substantial decomposition.

The high photoresistance of C-Naphox allowed continuous STED imaging (Figure 12). The fluorescence intensity of C-Naphox in HeLa cells remained virtually unchanged after recording five STED images. This is especially noteworthy, considering that under similar conditions, the signal intensity for Alexa-488-labeled cells decreased to only 6% of the initial value. Moreover, the intracellular fluorescence intensity of C-Naphox remained at 83% of the initial value even after recording 50 STED images.

A recent comparative study revealed that the high photostability of C-Naphox is not simply due to the rigidly bridged π -conjugated skeleton, but also to the steric shielding arising from the two phenyl groups at the methylene bridge.³⁰ However, a detailed rationalization of the high photostability should require further experimental investigations especially into the excited-state dynamics, which is currently in progress in our laboratory. Interestingly, we have also demonstrated that a silicon-bridged congener shows comparable photostability.³⁰

Figure 12. Repeated STED imaging of HeLa cells using a) C-Naphox and b) Alexa 488-conjugated anti-KDEL antibodies. Five images were recorded in intervals of 165 s; irradiation sources: a tunable white-light excitation laser (488 nm, 80 MHz, output power: 70%, AOTF 80%) and a CW-STED laser (592 nm CW laser, output power: 95%, AOTF 100%).

4.2 Development of PhoxBright 430 (PB430)

Although C-Naphox allows acquiring repeated STED images, the practical use of C-Naphox in cell imaging is still hampered by several drawbacks. For instance, its poor water-solubility induces non-specific binding to hydrophobic organelles, such as the endoplasmic reticulum. The decreased fluorescence quantum yields of C-Naphox in aqueous media are also less than desirable for some purposes. The absence of a bioconjugation site is another obstacle that needs to be circumvented in order to enable a multitude of biological applications. To address these issues, we designed the benzo[*b*]phosphole-based dye PhoxBright 430 (hereafter denoted as PB430) (Figure 13).³¹

Figure 13. Chemical structure of PB430 and a 3-D STED image of PB430-immunolabeled HeLa cell microtubules, including a color scale corresponding to the height (increments in z -direction: 50 nm; scale bar: 5 μm). Images were recorded under the following conditions: excitation at 470 nm (WLL, 5 μW), depletion at 592 nm (CW-STED, 30 mW; STED 3D z donut, 50%), and gated detection at $t_g=0.5$ ns.

PB430 exhibits improved hydrophilicity thanks to the presence of anionic side chains. In addition, the diphenylamino group in C-Naphox was replaced with an aryl group in PB430. The lack of intramolecular charge-transfer (ICT) character in the excited state imparts PB430 with a high fluorescence quantum yield ($\Phi_F = 0.67$), even in aqueous media. However, the removal of the electron-donating diphenylamino group also resulted in a substantial blue-shift of the absorption band to the UV region. Therefore, we decided to change the fusion mode of the naphthalene moiety in PB430, which gave rise to a red-shift of the absorption band where (absorption edge reached 480 nm). This modification enabled us to use a visible light laser (470 nm) for excitation, which is commonly used in STED microscopy.

PB430 moreover contains a functional group that allows bioconjugation to antibodies for immunolabeling. Thus, PB430 could be converted into the corresponding NHS ester, followed by conjugation with goat anti-mouse IgG antibodies. The thus prepared dye-conjugated antibodies retained exceptionally high photostability, which enabled us to perform multiple acquisitions of STED images without the need for a custom-built microscope or antifading agents. The fluorescence intensity of PB430-immunolabeled microtubules retained more than 50% of the initial intensity even after recording 30 images. Under identical STED conditions, Alexa Fluor 488 was completely photobleached after recording three pictures.

Based on the super-photostable characteristics of PB430, the construction of 3-D images using 2-D STED images was achieved. It should be noted that the construction of a 3-D image from 2-D STED images is challenging, as rapid photobleaching occurs during sequential xy -scans when using conventional dyes. We repeatedly scanned an area of PB430-labeled tubulins while changing the z -positions in increments of 50 nm. Figure 13 shows a 3-D image of the microtubules obtained based on 81 xy -images. The result clearly demonstrates the impact of the exceptional photoresistance of PB430.

The distinct photostability of PB430 also allowed applications in multi-color imaging, which is based on a photostability-based separation method.³²

5. Conclusion

Among various heterole rings, phosphole occupies a unique position, as its properties can be dramatically changed via chemical transformations of the phosphorus center. Phosphole P -oxide represents a particularly useful scaffold, given its high electron-accepting characteristics and chemical stability. Most of the conventional research on this skeleton has been directed toward applications in organic electronics. In contrast, our studies have shed light on a new aspect of phosphole chemistry, *i.e.*, applications of fluorescent benzophosphole derivatives in fluorescence bioimaging. The development of robust synthetic methods enabled us to produce a series of relevant derivatives and thereby conduct detailed studies on their structure-property relationships. Based on the knowledge thus accumulated, we were able to optimize the π -conjugated scaffold and finally obtained useful fluorescence dyes for bioimaging. Especially diphenylaminophenyl-substituted benzo[*b*]phosphole P -oxide exhibited a large solvatochromism of its fluorescence and great utility as an environment-polarity-sensitive probe. Moreover, its utility as a staining agent for lipid droplets is superior to the currently commercially available dyes. Furthermore, the fully ring-fused, structurally reinforced phosphole P -oxide derivatives C-Naphox and PB430 exhibit outstanding photostability and allowed acquiring multiple STED images without the need for a custom-built microscope or antifading agents. Using PB430-conjugated antibodies, we accomplished a 3-D reconstruction of super-resolution STED images as well as photostability-based multicolor STED imaging. These results open a new avenue for phosphole chemistry and materials-oriented main-group chemistry.

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References and Notes

- For example: (a) Yamaguchi, S.; Tamao, K. *Chem. Lett.* **2005**, *34*, 2. (b) Fukazawa, A.; Yamaguchi, S. *Chem. Asian J.* **2009**, *4*, 1386.
- (a) Crassous, J.; Réau, R. *Dalton Trans.* **2008**, 6865. (b) Baumgartner, T. *Acc. Chem. Res.* **2014**, *47*, 1613. (c) Stolar, M.; Baumgartner, T. *Chem. Asian J.* **2014**, *9*, 1212. (d) Matano, Y. *Chem. Rec.* **2015**, *15*, 636. (e) Duffy, M. P.; Delaunay, W.; Bouit, P.-A.; Hissler, M. *Chem. Soc. Rev.* **2016**, *45*, 5296. (f) Joly, D.; Bouit, P.-A.; Hissler, M. *J. Mater. Chem. C* **2016**, *4*, 3686. (g) Shameem, M. A.; Orthaber, A. *Chem. Eur. J.* **2016**, *22*, 10718. (h) Hibner-Kulicka, P.; Joule, J. A.; Skalik, J.; Bałczewski, P. *RSC Adv.* **2017**, *7*, 9194.
- Yamaguchi, E.; Wang, C.; Fukazawa, A.; Taki, M.; Sato, Y.; Sasaki, T.; Ueda, M.; Sasaki, N.; Higashiyama, T.; Yamaguchi, S. *Angew. Chem. Int. Ed.* **2015**, *54*, 4539.
- (a) Matano, Y.; Miyajima, T.; Fukushima, T.; Kaji, H.; Kimura, Y.; Imahori, H. *Chem. Eur. J.* **2008**, *14*, 8102. (b) Saito, A.; Miyajima, T.

- Nakashima, M.; Fukushima, T.; Kaji, H.; Matano, Y.; Imahori, H. *Chem. Eur. J.* **2009**, *15*, 10000. (c) Matano, Y.; Saito, A.; Fukushima, T.; Tokudome, Y.; Suzuki, F.; Sakamaki, D.; Kaji, H.; Ito, A.; Tanaka, K.; Imahori, H. *Angew. Chem. Int. Ed.* **2011**, *50*, 8016. (d) Matano, Y.; Ohkubo, H.; Honsho, Y.; Saito, A.; Seki, S.; Imahori, H. *Org. Lett.* **2013**, *15*, 932. (e) Hong, E. Y.-H.; Poon, C.-T.; Yam, V. W.-W. *J. Am. Chem. Soc.* **2016**, *138*, 6368.
- 5) (a) Fave, C.; Cho, T.-Y.; Hissler, M.; Chen, C.-W.; Luh, T.-Y.; Wu, C.-C.; Réau, R. *J. Am. Chem. Soc.* **2003**, *125*, 9254. (b) Su, H.-C.; Fadhel, O.; Yang, C.-J.; Cho, T.-Y.; Fave, C.; Hissler, M.; Wu, C.-C.; Réau, R. *J. Am. Chem. Soc.* **2006**, *128*, 983. (c) Tsuji, H.; Sato, K.; Sato, Y.; Nakamura, E. *J. Mater. Chem.* **2009**, *19*, 3364. (d) Fadhel, O.; Gras, M.; Lemaitre, N.; Deborde, V.; Hissler, M.; Geoffroy, B.; Réau, R. *Adv. Mater.* **2009**, *21*, 1261. (e) Chen, H.; Delaunay, W.; Li, J.; Wang, Z.; Bouit, P.-A.; Tondelier, D.; Geoffroy, B.; Mathey, F.; Duan, Z.; Réau, R.; Hissler, M. *Org. Lett.* **2013**, *15*, 330. (f) Gong, P.; Ye, K.; Sun, J.; Chen, P.; Xue, P.; Yang, H.; Lu, R. *RSC Adv.* **2015**, *5*, 94990. (g) Riobé, F.; Szűcs, R.; Bouit, P.-A.; Tondelier, D.; Geoffroy, B.; Aparicio, F.; Buendía, J.; Sánchez, L.; Réau, R.; Nyulászi, L.; Hissler, M. *Chem. Eur. J.* **2015**, *21*, 6547. (h) Zhou, Y.; Yang, S.; Li, J.; He, G.; Duan, Z.; Mathey, F. *Dalton Trans.* **2016**, *45*, 18308. (i) Zhuang, Z.; Bu, F.; Luo, W.; Peng, H.; Chen, S.; Hu, R.; Qin, A.; Zhao, Z.; Tang, B. Z. *J. Mater. Chem. C* **2017**, *5*, 1836.
 - 6) (a) Sung, M. J.; Kim, Y.; Lee, S. B.; Lee, G. B.; An, T. K.; Cha, H.; Kim, S. H.; Park, C. E.; Kim, Y.-H. *Dye. Pigm.* **2016**, *125*, 316. (b) Lee, J. E.; An, T. K.; Kim, Y.-H. *Macromol. Res.* **2016**, *24*, 629.
 - 7) (a) Tsuji, H.; Sato, K.; Sato, Y.; Nakamura, E. *Chem. Asian J.* **2010**, *5*, 1294. (b) Kira, A.; Shibano, Y.; Kang, S.; Hayashi, H.; Umeyama, T.; Matano, Y.; Imahori, H. *Chem. Lett.* **2010**, *39*, 448. (c) Matano, Y.; Saito, A.; Suzuki, Y.; Miyajima, T.; Akiyama, S.; Otsubo, S.; Nakamoto, E.; Aramaki, S.; Imahori, H. *Chem. Asian J.* **2012**, *7*, 2305. (d) Matano, Y.; Ohkubo, H.; Miyata, T.; Watanabe, Y.; Hayashi, Y.; Umeyama, T.; Imahori, H. *Eur. J. Inorg. Chem.* **2014**, 1620. (e) Kim, Y. J.; Kim, M.-J.; An, T. K.; Kim, Y.-H.; Park, C. E. *Chem. Commun.* **2015**, *51*, 11572. (f) Park, K. H.; Kim, Y. J.; Lee, G. B.; An, T. K.; Park, C. E.; Kwon, S.-K.; Kim, Y.-H. *Adv. Funct. Mater.* **2015**, *25*, 3991. (g) Bui, T. T. T.; Park, S.; Hoang, Q. V.; Song, C. E.; Lee, S. K.; Lee, J.-C.; Moon, S.-J.; Shin, W. S. *Synth. Met.* **2016**, *215*, 235.
 - 8) Öberg, E.; Appelqvist, H.; Nilsson, K. P. R. *Front. Chem.* **2017**, *5*, 28.
 - 9) (a) Blom, H.; Widengren, J. *Chem. Rev.* **2017**, *117*, 7377. (b) Erdmann, R. S.; Takakura, H.; Thompson, A. D.; Rivera-Molina, F.; Allgeyer, E. S.; Bewersdorf, J.; Toomre, D.; Schepartz, A. *Angew. Chem. Int. Ed.* **2014**, *53*, 10242. (c) Lukinavičius, G.; Reymond, L.; D'Este, E.; Masharina, A.; Göttfert, F.; Ta, H.; Güther, A.; Fournier, M.; Rizzo, S.; Waldmann, H.; Blaukopf, C.; Sommer, C.; Gerlich, D. W.; Arndt, H. D.; Hell, S. W.; Johnsson, K. *Nat. Methods* **2014**, *11*, 731. (d) Lukinavičius, G.; Umezawa, K.; Olivier, N.; Honigsmann, A.; Yang, G. Y.; Plass, T.; Mueller, V.; Reymond, L.; Jr, I. R. C.; Luo, Z. G.; Schultz, C.; Lemke, E. A.; Heppenstall, P.; Eggeling, C.; Manley, S.; Johnsson, K. *Nat. Chem.* **2013**, *5*, 132. (e) Nägerl, U. V.; Bonhoeffer, T. J. *Neurosci.* **2010**, *30*, 9341. (f) Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature* **2006**, *440*, 935.
 - 10) (a) Liphardt, B.; Liphardt, B.; Lüttke, W. *Opt. Commun.* **1981**, *38*, 207. (b) Altman, R. B.; Terry, D. S.; Zhou, Z.; Zheng, Q.; Geggier, P.; Kolster, R. A.; Zhao, Y.; Javitch, J. A.; Warren, J. D.; Blanchard, S. C. *Nat. Methods* **2012**, *9*, 68. (c) Grimm, J. B.; English, B. P.; Chen, J.; Slaughter, J. P.; Zhang, Z.; Revyakin, A.; Patel, R.; Macklin, J. J.; Normanno, D.; Singer, R. H.; Lionnet, T.; Lavis, L. D. *Nat. Methods* **2015**, *12*, 244. (d) Mitronova, G. Y.; Belov, V. N.; Bossi, M. L.; Wurm, C. A.; Meyer, L.; Medda, R.; Moneron, G.; Bretschneider, S.; Eggeling, C.; Jakobs, S.; Hell, S. W. *Chem. Eur. J.* **2010**, *16*, 4477. (e) Kolmakov, K.; Heibisch, E.; Wolfram, T.; Nordwig, L. A.; Wurm, C. A.; Ta, H.; Westphal, V.; Belov, V. N.; Hell, S. W. *Chem. Eur. J.* **2015**, *21*, 13344. (f) Sun, W.-C.; Gee, K. R.; Klaubert, D. H.; Haugland, R. P. *J. Org. Chem.* **1997**, *62*, 6469. (g) Boyarskiy, V. P.; Belov, V. N.; Medda, R.; Hein, B.; Bossi, M.; Hell, S. W. *Chem. Eur. J.* **2008**, *14*, 1784. (h) Panchuk-Voloshina, N.; Haugland, R. P.; Bishop-Stewart, J.; Bhalgat, M. K.; Millard, P. J.; Mao, F.; Leung, W. Y.; Haugland, R. P. *J. Histochem. Cytochem.* **1999**, *47*, 1179.
 - 11) (a) Hayashi, Y.; Matano, Y.; Suda, K.; Kimura, Y.; Nakao, Y.; Imahori, H. *Chem. Eur. J.* **2012**, *18*, 15972. (b) Matano, Y.; Hayashi, Y.; Suda, K.; Kimura, Y.; Imahori, H. *Org. Lett.* **2013**, *15*, 4458. (c) Matano, Y.; Motegi, Y.; Kawatsu, S.; Kimura, Y. *J. Org. Chem.*, **2015**, *80*, 5944.
 - 12) (a) Chen, Y.-R.; Duan, W.-L. *J. Am. Chem. Soc.* **2013**, *135*, 16754. (b) Unoh, Y.; Hirano, K.; Satoh, T.; Miura, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 12975. (c) Ma, W.; Ackermann, L. *Synthesis* **2014**, *46*, 2297.
 - (d) Hu, G.; Zhang, Y.; Su, J.; Li, Z.; Gao, Y.; Zhao, Y. *Org. Biomol. Chem.* **2015**, *13*, 8221. (e) Zhang, P.; Gao, Y.; Zhang, L.; Li, Z.; Liu, Y.; Tang, G.; Zhao, Y. *Adv. Synth. Catal.* **2016**, *358*, 138. (f) Quint, V.; Morlet-Savary, F.; Lohier, J. F.; Lalevee, J.; Gaumont, A. C.; Lakhdar, S. *J. Am. Chem. Soc.* **2016**, *138*, 7436. (g) Ma, D.; Chen, W.; Hu, G.; Zhang, Y.; Gao, Y.; Yin, Y.; Zhao, Y. *Green Chem.* **2016**, *18*, 3522.
 - 13) (a) Wu, B.; Santra, M.; Yoshikai, N. *Angew. Chem. Int. Ed.* **2014**, *53*, 7543. (b) Yoshikai, N.; Santra, M.; Wu, B. *Organometallics* **2017**, *36*, 2637.
 - 14) (a) Rausch, M. D.; Klemann, L. P. *J. Am. Chem. Soc.* **1967**, *89*, 5732. (b) Winter, W. *Tetrahedron Lett.* **1975**, *16*, 3913. (c) Winter, W. *Chem. Ber.* **1977**, *110*, 2168. (d) Butters, T.; Winter, W. *Chem. Ber.* **1984**, *117*, 990. (e) Märkl, G.; Jin, G. Y.; Berr, K.-P. *Tetrahedron Lett.* **1993**, *34*, 3103. (f) Tsuji, H.; Sato, K.; Iles, L.; Itoh, Y.; Sato, Y.; Nakamura, E. *Org. Lett.* **2008**, *10*, 2263. (g) Tsuji, H.; Sato, K.; Sato, Y.; Nakamura, E. *J. Mater. Chem.* **2009**, *19*, 3364. (i) Sanji, T.; Shiraishi, K.; Kashiwabara, T.; Tanaka, M. *Org. Lett.* **2008**, *10*, 2689. See also 7a.
 - 15) Fukazawa, A.; Hara, M.; Okamoto, T.; Son, E.-C.; Xu, C.; Tamao, K.; Yamaguchi, S. *Org. Lett.* **2008**, *10*, 913.
 - 16) (a) Yamaguchi, S.; Xu, C.; Tamao, K. *J. Am. Chem. Soc.* **2003**, *125*, 13662. (b) Xu, C.; Wakamiya, A.; Yamaguchi, S. *Org. Lett.* **2004**, *6*, 3707. (c) Xu, C.; Wakamiya, A.; Yamaguchi, S. *J. Am. Chem. Soc.* **2005**, *127*, 1638.
 - 17) Fukazawa, A.; Yamada, H.; Yamaguchi, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 5582.
 - 18) Fukazawa, A.; Yamaguchi, E.; Ito, E.; Yamada, H.; Wang, J.; Irle, S.; Yamaguchi, S. *Organometallics* **2011**, *30*, 3870.
 - 19) Fukazawa, A.; Yamada, H.; Sasaki, Y.; Akiyama, S.; Yamaguchi, S. *Chem. Asian J.* **2010**, *5*, 466.
 - 20) Fukazawa, A.; Ichihashi, Y.; Kosaka, Y.; Yamaguchi, S. *Chem. Asian J.* **2009**, *4*, 1729.
 - 21) (a) Vaillard, S.; Mück-Lichtenfeld, C.; Grimme, S.; Studer, A. *Angew. Chem. Int. Ed.* **2007**, *46*, 6533. (b) Bruch, A.; Ambrosius, A.; Fröhlich, R.; Studer, A.; Guthrie, D. B.; Zhang, H.; Curran, D. P. *J. Am. Chem. Soc.* **2010**, *132*, 11452. (c) Lamas, M.-C.; Studer, A. *Org. Lett.* **2011**, *13*, 2236.
 - 22) Bruch, A.; Fukazawa, A.; Yamaguchi, E.; Yamaguchi, S.; Studer, A. *Angew. Chem. Int. Ed.* **2011**, *50*, 12094.
 - 23) Greulich, T. W.; Suzuki, N.; Daniliuc, C. G.; Fukazawa, A.; Yamaguchi, E.; Studer, A.; Yamaguchi, S. *Chem. Commun.* **2016**, *52*, 2374.
 - 24) Greulich, T. W.; Yamaguchi, E.; Doerenkamp, C.; Daniliuc, C. G.; Fukazawa, A.; Eckert, H.; Yamaguchi, S.; Studer, A. *Chem. Eur. J.* **2017**, *23*, 6029.
 - 25) Reus, C.; Stolar, M.; Vanderkley, J.; Nebauer, J.; Baumgartner, T. *J. Am. Chem. Soc.* **2015**, *137*, 11710.
 - 26) Yamaguchi, E.; Fukazawa, A.; Kosaka, Y.; Yokogawa, D.; Irle, S.; Yamaguchi, S. *Bull. Chem. Soc. Jpn.* **2015**, *88*, 1545.
 - 27) (a) Bagatolli, L. A. *Biochim. Biophys. Acta* **2006**, *1758*, 1541. (b) Demchenko, A. P.; Mély, Y.; Duportail, G.; Klymchenko, A. S. *Biophys. J.* **2009**, *96*, 3461. (c) Klymchenko, A. S.; Kreder, R. *Chem. Biol.* **2014**, *21*, 97. (d) Loving, G. S.; Sainlos, M.; Imperiali, B. *Trends Biotechnol.* **2010**, *28*, 73.
 - 28) Taki, M.; Ogasawara, H.; Osaki, H.; Fukazawa, A.; Sato, Y.; Ogasawara, K.; Higashiyama, T.; Yamaguchi, S. *Chem. Commun.* **2015**, *51*, 11880.
 - 29) Wang, C.; Fukazawa, A.; Taki, M.; Sato, Y.; Higashiyama, T.; Yamaguchi, S. *Angew. Chem. Int. Ed.* **2015**, *54*, 15213.
 - 30) Adler, R. A.; Wang, C.; Fukazawa, A.; Yamaguchi, S. *Inorg. Chem.* **2017**, *56*, 8718.
 - 31) Wang, C.; Taki, M.; Sato, Y.; Fukazawa, A.; Higashiyama, T.; Yamaguchi, S. *J. Am. Chem. Soc.* **2017**, *139*, 10374.
 - 32) Rönnlund, D.; Xu, L.; Perols, A.; Gad, A. K. B.; Karlström, A. E.; Auer, G.; Widengren, J. *ACS Nano* **2014**, *8*, 4358.

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